Blueberry Red Ringspot Observations and Findings in North Carolina

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Abstract

Observation of suspected blueberry red ringspot virus (BRRV) prompted a survey of the 50-acre NCSU Ideal Tract blueberry farm, and of commercial farms in surrounding counties. Polymerase chain reaction (PCR) testing with red ringspot-specific primers was used to confirm the presence of the virus. Over 18,000 plants were surveyed visually, with PCR used for backup confirmation. The virus was detected primarily in highbush and southern highbush (*Vaccinium corymbosum*) seedlings, selections and cultivars. Incidence was low. Spread occurred primarily by propagation, but rare, widely scattered infections in older fields suggested an infrequent or inefficient winged vector. Hundreds of rabbiteye (*V. virgatum*, syn. *V. ashei*) bushes were also surveyed; symptoms were not observed on any rabbiteye cultivars, and were observed but not confirmed on rabbiteye seedlings. Samples from the rabbiteye 'Columbus' were negative in PCR testing. Symptoms were observed, and confirmed by PCR, on highbush and southern highbush from three surrounding counties, including wild or feral blueberries near commercial fields. Symptoms were first seen in early June, became progressively more noticeable through August, and included red rings on leaves, stems and fruit. Scattered to numerous red rings (3-6 mm) with green or pale green centers were the most frequent symptom on leaves, usually visible only on the upper leaf surface. Larger (5-15 mm) red rings or spots were visible on stems of the current season's growth. Most cultivars/clones had few or no fruit symptoms; only 'Ozarkblue' produced distorted, unmarketable fruit on infected bushes. Some infected seedlings and selections appeared to be stunted. The virus has also been reported from Georgia; in this study, southern highbush cultivars 'Star' and 'Misty', reportedly from a Georgia nursery source, were symptomatic and tested positive for the virus. Studies beginning in 2008 will assess possible seed transmission, possible vectors, and determine incidence and severity in the southeastern US.

INTRODUCTION

Blueberry red ringspot virus (BRRV) was first reported as a graft-transmissible virus by Hutchison and Varney in 1954. The disease has been observed in several US states (Ramsdell, 1995). Symptoms are difficult to see during winter, spring and early summer, but become more visible in late summer and early fall. Symptoms include red rings on stems, fruit and leaves (Figs. 1-4). Red rings or spots measuring 5-15 mm and occasionally larger are seen on stems of the current season's growth (Fig.1). Scattered to crowded red rings (3-6 mm) with green or pale green centers are the most frequent symptom on leaves, and, unlike fungal leaf spots, are usually visible only on the upper leaf surface (Fig. 2). Circular blotches, pale spots, or distortions may also be visible on

ripening fruit (Fig. 3-4) though yield is often not affected. Plants are infected for life, and cuttings taken from infected plants will also have the disease. Aphids and mealybugs have been proposed as possible vectors, but attempts to demonstrate vector transmission have been unsuccessful (Ramsdell, 1995).

This study was initiated in 2007 following observations of suspected BRRV at the NCSU Horticultural Crops Research Station in Castle Hayne. The four objectives of this work were to (1) verify the preliminary diagnosis of BRRV, (2) determine the extent of viral infection among the diverse species and interspecific clones at this location, (3) collect evidence on how the virus was spread (whether by propagation or by insect vectors) and (4) begin assessing the incidence and severity of BRRV in southeastern North Carolina and the southeastern United States.

MATERIALS AND METHODS

All blueberry bushes (18,000+) at the Ideal Tract, NCSU Horticultural Crops Research Station in Castle Hayne, NC were surveyed visually, and symptomatic bushes were flagged for removal. Representative samples were taken from symptomatic and non-symptomatic bushes for PCR testing. Fields were mapped to show the location of each infected bush, and these observations were correlated with maps of species, selections, or seedling populations.

Although a systematic survey of the state's blueberry industry was not conducted in 2007, observations were made during routine farm visits to commercial fields and at other research station sites. Samples were collected from symptomatic bushes in these fields and in adjacent areas of feral/wild stands.

Sampling and testing

Samples of leafy stems from the current season's growth, with leaves still attached, were collected between June and October 2007. A typical sample from a single bush consisted of three to four leafy stem pieces of varying age (i.e., mature, intermediate and immature growth flushes), with each stem piece being approximately 10 cm long and bearing four to six leaves. Samples were immediately placed in plastic bags on ice and refrigerated (5-7°C) until shipped to the USDA/ARS lab, usually within 24-48h. Samples were subjected to PCR testing using BRRV-specific primers. Most samples were frankly symptomatic, but some had atypical or faint symptoms. Symptomless plants from the same fields were tested as controls. Control testing also included greenhouse-grown plants from tissue culture that had not been exposed to outdoor conditions or to infected plants.

DNA extraction and PCR

DNA was extracted from blueberry leaf tissue using the CTAB procedure (Stewart and Via, 1993) as modified by Novy and Vorsa (1995) except that 200 mg of plant tissue was ground at room temperature in a 4 x 5 inch mesh bag (Agdia, Elkhart, IN) containing 2 ml of CTAB buffer using a circular bearing tissue homogenizer (Agdia) attached to a drill press. The amounts of other reagents used in the extraction procedure were increased in proportion to the volume of CTAB buffer used. Purified DNA was quantified using a fluorometer (DyNA Quant 200, Hoefer Scientific Instruments, San Francisco, CA). The same procedure was used to extract DNA from bark scrapings of one-year old stems of blueberry.

Red ringspot-specific primers were designed based on the published BRRV sequence (Glasheen et al., 2002, GenBank accession AF404509) with the aid of the PrimerSelect module in the Lasergene (DNASTAR Inc., Madison, WI) software package. The expected size of the amplified BRRV fragment is 549 bp. HotMaster Taq polymerase (Eppendorf, Westbury, NY) was used for all amplifications. The program used for amplification was: 94°C for 2 min (initial denaturation), followed by 30 cycles of 94°C for 20 sec, 57°C for 10 sec and 70°C for 45 sec, with a final extension at 70°C for 5 min. All PCR reactions contained 1X reaction buffer (supplied by the manufacturer), 200 μM

dNTP's, 1 µM of each primer, 1U of polymerase and ~ 50 ng of plant DNA extract in a final 25 µl reaction volume. PCR reactions were performed on a GeneAmp 9700 PCR System (Applied Biosystems, Foster City, CA). A portion (usually 10 µl) of each reaction was run on a 0.8% agarose gel and stained with ethidium bromide to visualize the amplified fragments.

RESULTS

The virus was observed primarily in highbush (*Vaccinium corymbosum*) and southern highbush seedlings, selections and cultivars, and was confirmed by PCR (Table 1). Incidence was low overall, but high in individual clones (data not shown) suggesting that spread occurred primarily by propagation. However, rare, widely scattered infections in older fields suggested an infrequent or inefficient winged vector. Hundreds of rabbiteye (*V. virgatum*, syn. *V. ashei*) bushes were also surveyed; symptoms were not observed on any named rabbiteye cultivars, and were observed but not confirmed on rabbiteye seedlings. Samples from the rabbiteye 'Columbus' were negative in PCR testing. Symptoms were observed, and again confirmed by PCR, from highbush and southern highbush blueberries in three surrounding counties, including wild or feral blueberries adjacent to commercial fields (Table 2). Most cultivars and clones had few or no fruit symptoms, and fruit symptoms faintly visible on ripening berries disappeared as the berries became fully ripe; only 'Ozarkblue' produced distorted, unmarketable fruit on infected bushes. Some infected seedlings and selections appeared to be stunted (data not shown). Plants of two southern highbush cultivars reportedly obtained from a Georgia nursery source were symptomatic, and tested positive for the virus (Table 2).

PCR techniques and primers gave positive reactions for BRRV in 31 of 33 visibly infected samples. No positive reactions were detected among asymptomatic control samples.

CONCLUSIONS

Blueberry red ringspot has long been known to occur in North Carolina, but was not commonly observed, and not thought to spread rapidly in the field. Our results demonstrate that while this may yet be true, the potential for spread by propagation is a real threat. NC growers often propagate by hardwood cuttings, at a time of the year when symptoms are not visible. Even those who collect leafy softwood cuttings in late summer may not be aware of the symptoms of BRRV, and may thus unknowingly take cuttings from visibly infected plants. To counter this possibility, our work has led to the production of a scouting guide to familiarize growers with the symptoms of this disease (Cline, 2008).

Blueberry red ringspot virus poses a significant threat to the NC blueberry industry. The necessity of roguing out infected bushes has already depleted valuable breeding lines. This study has demonstrated the risk of spread via plant propagation, or by as-yet-unknown vectors. The effects of the virus on new, diverse interspecific crosses characterized by newer southern highbush cultivars may make it difficult for growers to identify and isolate infected plants. For instance, 'Ozarkblue' shows highly visible symptoms, while symptoms are quite easy to overlook on most other SHB clones. Studies beginning in 2008 will assess possible seed and pollen transmission, possible vectors, and will attempt to determine incidence and severity in the southeastern US.

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Tables

Table 1. Incidence of BRRV symptoms in fields A2, A3, B1, B2, B3, C1 and D1 at the NCSU Horticultural Crops Research Station (Ideal Tract) in Castle Hayne.

Blueberry type/species	No. bushes surveyed	No. with BRRV symptoms	Percent affected	Confirmed by PCR
Highbush cultivars Southern highbush cultivars *Vaccinium corymbosum* L.	3,918	63	1.6%	yes
Rabbiteye cultivars and seedlings *Vaccinium virgatum* Ait. (Syn. *V. ashei* Reade)	3,102	23	0.7%	no
Other *Vaccinium* species, highbush selections, interspecific hybrids and seedling populations	10,985	500	4.6%	yes

Table 2. Results of PCR testing on symptomatic and asymptomatic blueberry cultivars, selections and wild/feral clones from samples collected in 2007.

Cultivar/clone	Type[z]	Location	Date sampled	Symptoms	PCR
Arlen	SHB	Ideal Tract, field A-3	19Jul07	+	+
Bladen	SHB	Ideal Tract, field C-2		-	-
Bladen	SHB	Ideal Tract, field C-2		+	+
Columbus	R	Ideal Tract, field B-1		-	-
Craven	SHB	Ideal Tract, field B-1		+	+
Craven	SHB	Ideal Tract, field B-1		+	+
Craven	SHB	Ideal Tract, field B-2		-	-
Craven	SHB	Ideal Tract, field B-2		+	+
Croatan	HB	Ideal Tract, field B-1		-	-
Croatan	HB	Ideal Tract, field B-1		+	+
New Hanover	SHB	Ideal Tract, field E-3		+	-
New Hanover	SHB	Ideal Tract, field E-3		-	-
Palmetto	SHB	Ideal Tract, field A-3		-	-
US 508	HB	Ideal Tract, field E-3		-	-
US 508	HB	Ideal Tract, field E-3		-	-
NC 3104 (B)	SHB	Greenhouse/MPU		-	-
NC 4124 (A)	SHB	Greenhouse/MPU		-	-
NC 4443	SHB	Greenhouse/MPU		-	-
NC 4126 (B)	SHB	Greenhouse/MPU		-	-
NC 3478 (B)	SHB	Greenhouse/MPU		-	-
Carteret (M)	SHB	Greenhouse/MPU		-	-
NC 2849 (A)	SHB	Greenhouse/MPU		-	-
NC 4126 (A)	SHB	Greenhouse/MPU		-	-
NC 4126 (A)	SHB	Greenhouse/MPU		-	-
NC 3436 (A)	SHB	Greenhouse/MPU		-	-
NC 4131 (E)	SHB	Greenhouse/MPU		-	-
Pamlico (D)	SHB	Greenhouse/MPU		-	-
New Hanover (L)	SHB	Greenhouse/MPU		-	-
Ozarkblue	SHB	Bladen County	1Aug07	+	+
Ozarkblue	SHB	Bladen County		-	-
Duke	HB	Bladen County		+	+
Duke	HB	Bladen County		-	-
Star	SHB	Bladen County		+	+
Wild/feral	HB?	Columbus County	28Aug07	+	+
Wild/feral	HB?	Columbus County		-	-
O'Neal	SHB	Columbus County		+	+
Reveille	SHB	Pender County		+	+
Star	SHB	Pender County		+	-
New Hanover	SHB	Ideal Tract, field A-3		-	-
Carteret	SHB	Ideal Tract, field A-3		-	-
Star	SHB	Ideal Tract, field A-3		-	-
Star (hwd cutting)	SHB	Pender County		+	+
Star	SHB	Bladen County		-	-
NC selection	SHB	Fletcher, NC 1(36)		+	+
NC 4812	SHB	Fletcher, NC 4(30)		+	+
NC 4812	SHB	Fletcher, NC 4(35)		+	+
NC selection	SHB	Fletcher, NC 5(20)		+	+
NC 3464	SHB	Fletcher, NC 6(38)		+	+

Vaccinium sp.	?	Ideal Tract		+	+
Vaccinium sp.	?	Ideal Tract		+	+
Vaccinium sp.	?	Ideal Tract		+	+
Wild/feral	HB?	Pender County		+	+
Reveille	SHB	Pender County		+	+
Duke	HB	Bladen County		+	+
Star	SHB	Bladen County		+	+
Star (GA source)	SHB	Pender County		+	+
Misty (GA source)	SHB	Pender County		+	+
Duplin	SHB	Greenhouse/MPU		-	-
Duplin	SHB	Original mother plant		-	-
Pamlico (D)	SHB	Greenhouse/MPU		-	-
NC 1567	R	Original mother plant		-	-
NC 4339	SHB	Greenhouse/MPU		-	-
NC3104 (C)	SHB	Greenhouse/MPU		-	-
68-6 #1 (wild)	HB	Marlboro County, SC	9Oct07	-	-
68-6 #2 (wild)	HB	Marlboro County, SC		-	-
68-6 #3 (wild)	HB	Marlboro County, SC		-	-
68-6 #4 (wild)	HB	Marlboro County, SC		-	-
Wood's Bay (wild)	HB	Sumter County, SC		-	-
Sandhills (wild)	HB	Montgomery County		-	-
So. Pines (wild)	HB	Moore County		-	-
Wild	HB	Ashe County (WNC)		-	-
NC 2492	-			-	-
NC 2874	-			+	+
NC 4011	SHB			+	+
NC 4115	SHB			+	+
NC 4360	SHB			-	-
NC 4429	SHB			-	-
NC 4725	SHB			-	-
NC 4812	SHB			-	-
NC 4887	SHB			-	-
NC 4900	SHB			-	-
New Hanover	SHB			-	-
Reveille	SHB			+	+
SHF4A-8:6	SHB			-	-
Sunrise	HB			-	-
US 508	HB			-	-
Pisgah Inn (wild)	HB	Buncombe County		-	-
Pisgah Inn (wild)	HB	Buncombe County		-	-
Pisgah Inn (wild)	HB	Buncombe County		-	-
Pisgah Inn (wild)	HB	Buncombe County		-	-

[z]SHB = Southern highbush, HB = Highbush, R = Rabbiteye

Figures

Fig. 1. Blueberry red ringspot symptoms on stems of an unidentified highbush selection.

Fig. 2. Blueberry red ringspot (BRRV) symptoms on leaves. Note lack of spots on underside of leaf at center (unidentified highbush selection).

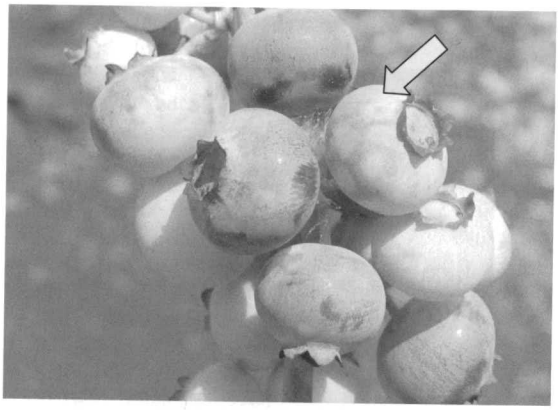

Fig. 3. Faint green spots (arrow) on 'Duke' caused by BRRV.

Fig. 4. Severely distorted fruit is rare, so far only seen on 'Ozarkblue' infected with BRRV.